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Effects of Thyroxine on Locomotor Activity and Carbon Dioxide Release in the Toad, *Bufo japonicus*

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ABSTRACT—To elucidate a role of thyroxine (T_4) in toad migration during the breeding season, we studied effects of administration of T_4 on locomotor activity and CO_2 release using normal and thyroidectomized adults of both sexes which were captured before hibernation. All the experiments were performed during the breeding season under laboratory conditions. Locomotor activity was estimated by passage of infrared beams in an activity box and CO_2 release was estimated comparing CO_2 contents in inflow air to the activity box and outflow air from the activity box. Locomotor activity was significantly suppressed (to 9%) by T_4 treatment (10 μg/day for 2 weeks) in intact males. T_4 also suppressed CO_2 release in intact males at moving states, while T_4 enhanced CO_2 release at resting states. T_4 had no effect on either locomotor activity or CO_2 release in female toads. Thyroidectomy in males resulted in a 3-fold increase in locomotor activity and T_4 administration suppressed activity in dose-dependent fashion (1 to 100 ng/g BW/day for 3 weeks). Neither thyroidectomy nor T_4 administration had any effect in females. These results suggest that thyroxine can not be the factor which induces breeding migration to the pond in the toad. If the sedative effect of thyroxine is physiological, it is probable that thyroxine initiates the post-breeding inactive stage.

INTRODUCTION

Thyroxine has been shown to play some role in the migration of lower vertebrates as reported in sticklebacks [1], young salmonids [1–4], tiger salamanders [5] and red-spotted newts [6]. Dent [6] provided evidence suggesting that thyroxine initiates the migration of post-breeding adult newts from water to land. One of the other proposed major roles of thyroxine is control of energy metabolism. In adult anurans, thyroid hormone administration stimulated the O_2 consumption of the animal [7–10] and glycogen metabolism in the liver [7, 11, 12].

We surveyed the annual cycles of plasma thyroid hormone levels in the toad, *Bufo japonicus*, and theorized two possible roles of thyroid hormone in the toad in winter and early spring [13]. They are initiation of migratory movement to and/or from the pond and regulation of energy metabolism at low temperatures during the breeding season.

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In the present experiment, we observed effects of administration of thyroxine on locomotor activity and CO_2 release to examine a relation between circulating thyroxine and breeding migration.

MATERIALS AND METHODS

Material

Adult male and female toads (*Bufo japonicus*) were captured in the suburbs of Tokyo in October and November, 1984 (Experimen I) and 1986 (Experiment II). The mean of their body weights and standard error was 203.8±9.5 g in Exp. I, and 127.4±6.2 g in Exp. II. Males and females were put in separate plastic boxes (55×40×43 cm) with loose fitting tops and kept outdoors. Wet pieces of plastic sponge were put in the boxes with the toads to maintain humidity. No feeding took place, since toads abstain from food during winter and spring.

Design of Experiment I

Seven females and nine males were used. Ten μg of L-thyroxine (SIGMA) suspended in saline

was injected daily for two weeks into the dorsal lymphsacs of four males and four females. The remaining three females and five males received injections of saline alone and served as controls. All the injections were performed between 0900 and 1100 hr. Locomotor activity and CO_2 release were measured for 18 hr from 1500 to 0900 hr the next morning during the period between March 3rd and 28th, 1985.

Design of Experiment II

Twenty-nine female and twenty-four male toads were used. Twenty-three females and nineteen males were thyroidectomized under anesthetization with MS-222 two weeks before the start of thyroxine treatments. A part of the hyoid cartilage was also removed with the thyroid. The remaining six females and five males were sham-operated. The thyroidectomized females were divided into four groups of 5, 6, 6, and 6, and received daily injections of 0, 0.001, 0.01, and 0.1 μg/g body weight/day of L-thyroxine in 0.1 ml of saline, into the dorsal lymphsac. The injections were performed once a day between 1000 and 1200 hr for three weeks until the day before the locomotor activity measurement. Thyroidectomized males were also divided into four groups of 5, 5, 4, and 5, and received the same injections as females. Locomotor activity of each toad was measured for 20 hr from 1200 to 0800 hr the next morning during the period between February 9th and March 2nd, 1987.

Recording of locomotor activity and CO_2 release in Experiment I

A small plastic chamber, 42 cm long, 20 cm wide and 15 cm deep (Fig. 1) was used to measure both the locomotor activity and CO_2 release of a toad simultaneously and automatically. Each toad was kept in the chamber for 21 hr (1200 to 0900 hr the next morning). After an initial three-hour acclimation, the locomotor activity and CO_2 release were continuously recorded for 18 hr. The temperature of the chamber was regulated at 10± 1°C. The chamber was illuminated from 0600 to 1800 hr, and kept in darkness the remaining hours. To quantify the locomotor activity of the toad in the chamber, seven pairs of photosensor uints

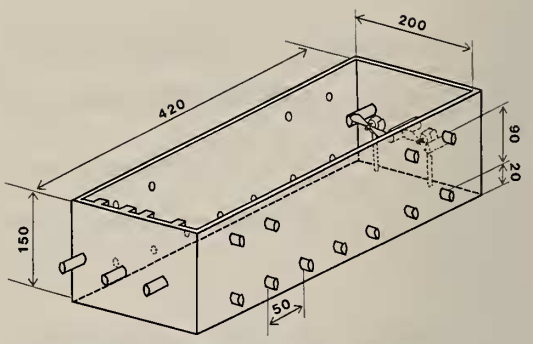


FIG. 1. The chamber used in Experiment I in order to record both the locomotor activity and the CO_2 release of a toad simultaneously and automatically. See text for details.

were mounted on the longitudinal side walls of the chamber at 5 cm intervals, 2 cm from the floor (Fig. 1). Each photosensor unit consisted of a infrared LED lamp (TLN 110, Toshiba) and a photodiode (TPS 703A, Toshiba). Interruption of the infrared light was recorded for each photosensor unit separately at 5 second intervals, and the records were stored in the memory of an 8 bit personal computer (NEC PC-8001, Fig. 2). Thus, the longitudinal position of the toad was recorded every 5 sec with the precision of ±2.5 cm. At the end of each experiment, data in the memory were transferred to a floppy disk. The total distance of locomotion was calculated later by the same computer.

Carbon dioxide released from a toad placed in the chamber was quantified as follows. The inflow air tube was divided into three parts and had 1.0 cm openings on the wall of one of the longitudinal ends of the chamber. The outflow air tube was connected similarly to the openings on the wall of the other end. Inflow air, which had been collected from the outdoors and stored in a balloon , was pumped into the chamber at the flow rate of 5.0 l per min. It was humidified by being passed through a water filter inserted between the balloon and the chamber. Air in the chamber was circulated by two small, slowly-rotating electric fans (RF-510T, Mabuchi) which were installed on a wall of the chamber (Fig. 1). The outflow air was channeled into an open-flow infrared gas analyzer (VIA-300, Horiba), and the CO_2 concentration was determined (Fig. 2). At the same time, part of

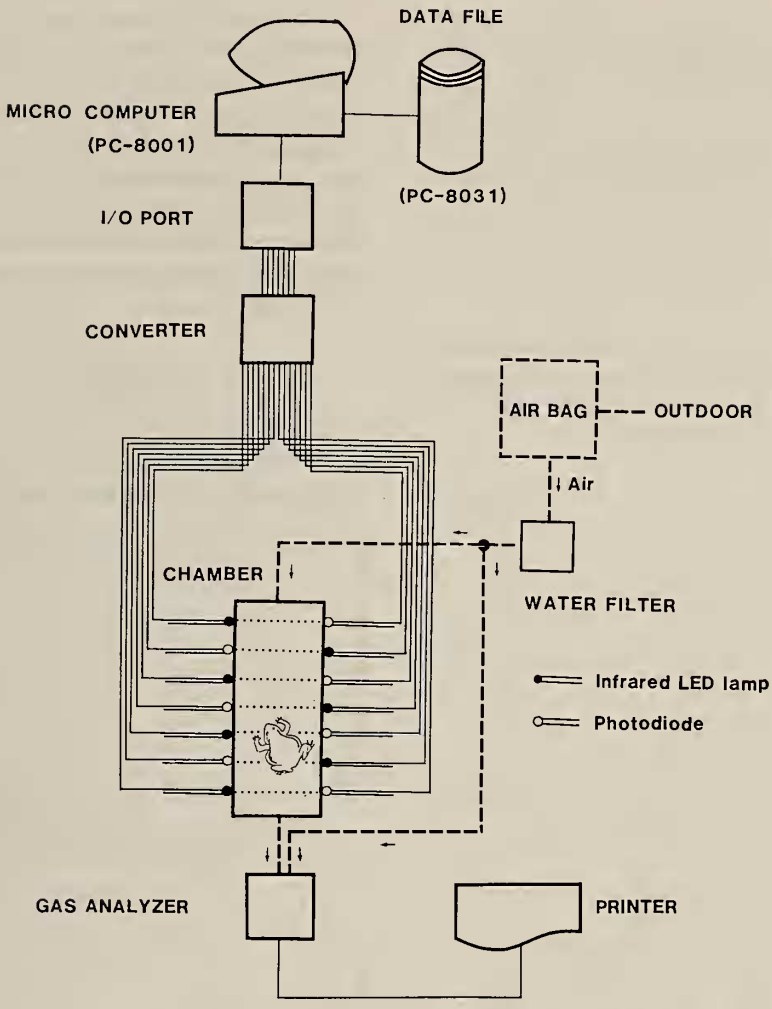


FIG. 2. Diagram showing the recording system in Experiment I.

the inflow air was introduced into the analyzer through a bypass, and its CO_2 concentration was also determined. From the difference in CO_2 concentration between the inflow and outflow air and the flow rate, the release of CO_2 from the toad was calculated. The mean CO_2 release when the toad stayed immobile at least one hour was referred to as the basal CO_2 release (Fig. 5). The mean difference between the active phase CO_2 release, which is the CO_2 release when the toad is moving, and the basal CO_2 release was referred to the activated CO_2 release (Fig. 5). The activated CO_2 release can be regarded as the rise in CO_2 release caused by locomotion.

Recording of locomotor activity in Experiment II

In this experiment, only the locomotor activity was measured. The chamber used had dimensions of $30\times30\times15$ cm (Fig. 3). The position of the toad in the chamber was recorded two-dimensionally by eight photosensor units. Each of the four walls was mounted with two infrared LED lamps and two photodiodes which were arranged reciprocally at 6 cm intervals. Their height from the floor was 2 cm on two opposing walls, and 4 cm on the other two. The air temperature and humidily of the chamber were regulated at 9.3 ± 0.7°C and 54 $\pm3\%$, respectively. The chamber was illuminated

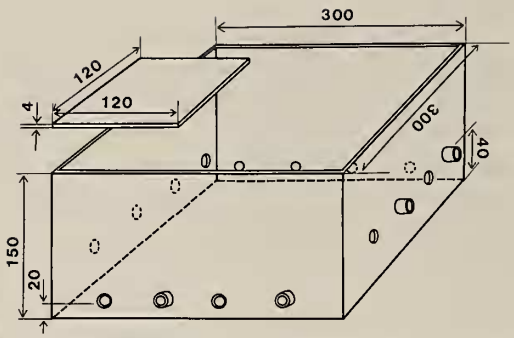


FIG. 3. The chamber used for recording the locomotor activity of a toad in Experiment II. The position of the toad in the chamber is recorded two dimensionally. See text for details.

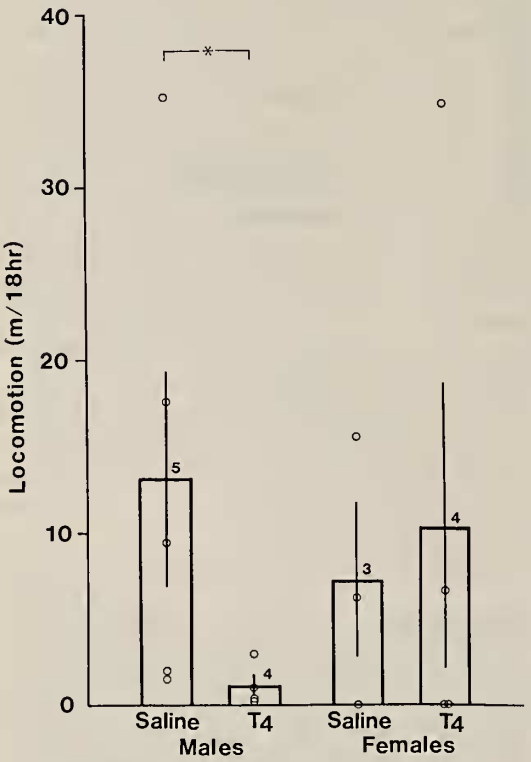


FIG. 4. Total locomotion distances (open circles) of thyroxine and saline-injected normal toads of both sexes for 18 hr. The column and vertical bar indicate the mean and standard error of each group, respectively. The mean of the thyroxine injected male group is significantly lower than that of the control group. No significant difference was observed between the female groups ($p>0.05$) when given the randomization test (*$p=0.0317$ by the randomization test).

from 0630 to 1730 hr, and kept in darkness the remaining hours. Data were recorded and analyzed as in Experiment I.

Statistical methods The significant difference between the means of the two groups was determined by the randomization test in Experiment I. The one-way matrix analysis of variance followed by Duncan's multiple range test was used in Experiment II. For these tests, computer programs [14] were employed.

RESULTS

Experiment I

The locomotor activity (total distance of

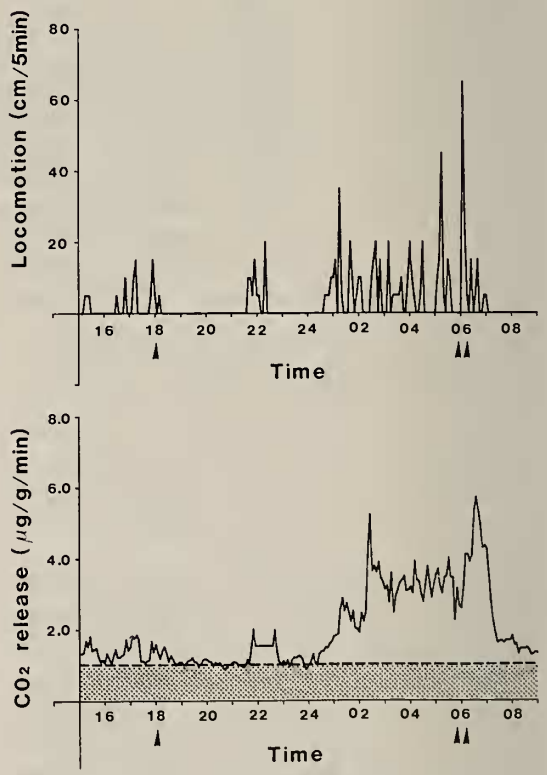


FIG. 5. Record of the locomotion distance (upper) and CO_2 release (lower) of a thyroxine-injected female toad (a typical case). Lights were turned on at 0600 hr (double arrow heads) and off at 1800 hr (single arrow head). Note that the CO_2 release is synchronized with the locomotion. In the lower figure, the dotted area corresponds to the basal CO_2 release and the area above the dotted line corresponds to the activated CO_2 release.

locomotion) of toads varied individually over a wide range (Fig. 4). Treatment with thyroxine seemed to have no effect on the mean locomotor activity of female toads, as the difference between the means of the control and treated groups (7.22 $\pm$4.52 m and 10.36$\pm$8.28 m, respectively) was not significant ($p>0.05$). However, in males, thyroxine suppressed activity, as the difference between the means of the control and treated groups (13.16$\pm$6.24 m and 1.14$\pm$1.61 m, respectively) was significant ($p<0.05$).

The change in CO_2 release faithfully coincided with changes in locomotor activity (Fig. 5). In females, there was no significant difference between activated CO_2 release of the control (377$\pm$277 ng/g B.W./min) and treated (259$\pm$170 ng/g B.W./min) groups (Fig. 6). In males, the activated CO_2 release in the control and treated groups was 532$\pm$175 ng/g B.W./min and 188$\pm$39 ng/g B.W./min, respectively, and the difference was significant ($p<0.05$, Fig. 6).

The basal CO_2 release was higher in females than in males. In females, it was not significantly changed by thyroxine treatment (Fig. 7). In males, however, the basal CO_2 release was significantly increased by thyroxine treatment, up to or over the levels of female toads ($p<0.05$, Fig. 7).

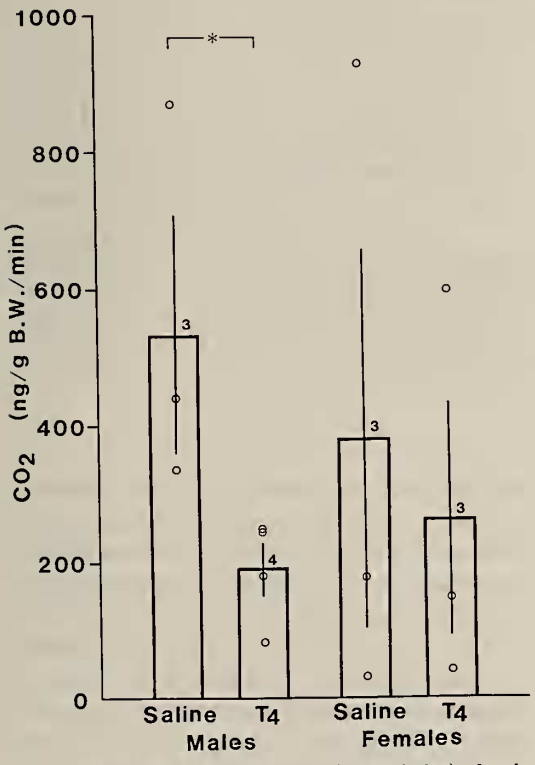


Fig. 6. The activated CO_2 release (open circles) of male and female toads. The column and vertical bar indicate the mean and standard error of each group, respectively. The mean of the thyroxine-injected male group is significantly lower than that of the control group (*p=0.0286 by the randomization test).

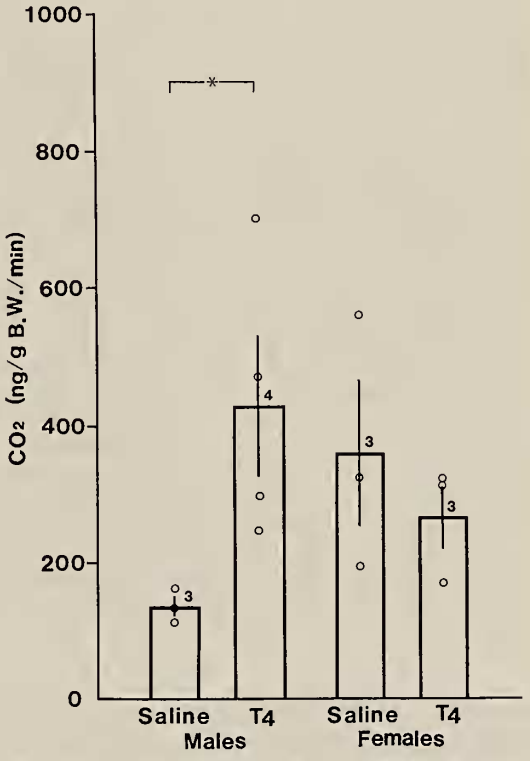
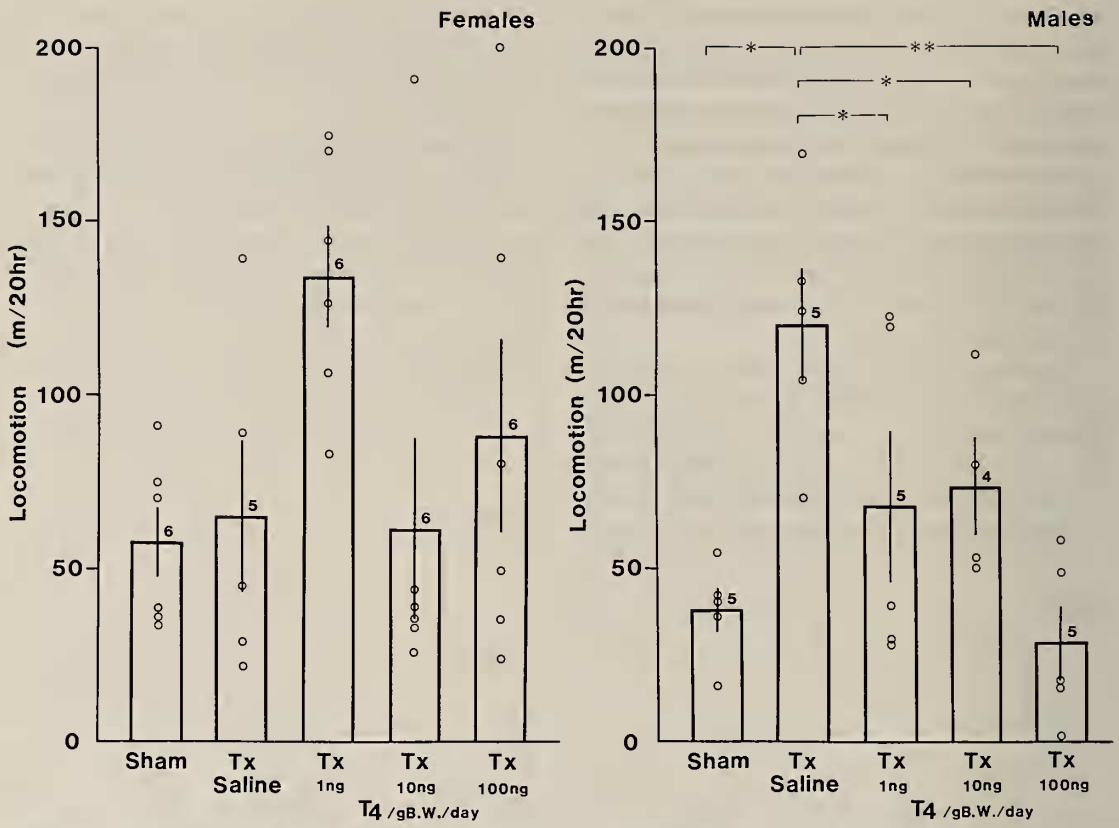


Fig. 7. The basal CO_2 release of male and female toads. The column and vertical bar indicate the mean and standard error of each group, respectively. The mean of the thyroxine-injected male group was significantly higher than that of its control group (*p=0.0286 by the randomization test).

Experiment II

In females, neither thyroidectomy nor thyroxine administrations seemed to influence locomotor activity, as the difference in mean activity among the five groups was not significant when tested by analysis of variance ($p>0.05$, Fig. 8). The group



FIGS. 8 and 9. Total locomotion distances (open circles) of sham-operated, thyroidectomized, and thyroidectomized and thyroxine-treated female (Fig. 8, Left) and male (Fig. 9, Right) toads. The column and vertical bar indicate the mean and standard error of each group, respectively. Thyroidectomized males showed a significantly higher ($p<0.01$ when given Duncan's multiple range test) locomotor activity than the shamoperated males. Replacement therapy suppressed the activity significantly ($p<0.01$ for the highest dose and $p<0.05$ for the lowest and middle doses when given Duncan's multiple range test).

receiving the lowest does of thyroxine had a higher mean activity level than the other groups, but this could be within the range of random fluctuation.

In contrast to females, thyroidectomized males showed significantly higher ($p<0.01$ by Duncan's multiple range test) locomotor activity than the sham-operated males, increase being about three-fold (Fig. 9). Replacement therapy suppressed the activity to some extent or even to a subnormal level depending upon the dose levels.

DISCUSSION

It is well known that prolactin is a factor which induces migration of newts and salamanders from land to water for breeding [15–20]. Recently however, Yoneyama *et al.* [21], Ishii *et al.* [22] and Yamamoto *et al.* [23] presented evidence showing that prolactin can not be the factor inducing migration to the breeding pond, at least in *Bufo*. Our survey of the annual cycle of plasma thyroid hormone levels in the toad, *Bufo japonicus*, revealed that the plasma thyroxine level increased gradually during the inactive winter period and reached a relatively high level at the commencement of the breeding migration. From this observation, we previously postulated that thyroxine, instead of prolactin, is the factor which induces breeding migration. However, in the present study, we found that both endogenous and

exogenous thyroxine suppressed the locomotor activity of male toads in spring, but we failed to show that effect in female toads. In either case, it is difficult to suggest that thyroxine is a suitable candidate for the migration inducing factor in the toad.

Dent [6] proposed the hypothesis that thyroxine causes the movement of terrestrial species of amphibians from water to land after breeding. Our recent finding [13] that the plasma thyroid hormone level in the toad is remarkably elevated when they arrive at the breeding pond strongly supports Dent's hypothesis. However, our present finding showing the sedative effect of thyroxine on locomotor activity is neutral to or may contradict Dent's hypothesis. This effect of thyroxine can however, explain the commencement of the post-breeding inactive period of the toad which lasts until May or June. Recently, Kubokawa and Ishii [24], surveying the annual cycle of various endocrine and metabolic parameters of the toad, pointed out that among various hormones, only thyroxine is secreted in the post-breeding inactive period. Further study is needed to elucidate the hormonal mechanism controlling the migration of toads to and from the breeding pond.

From many years past, it has been repeatedly reported that thyroxine stimulates O_2 consumption in whole animals [7, 8, 25] or liver slices in amphibians [9, 10, 26] as well as in higher vertebrates. In the present study, we observed that the basal CO_2 release in the male toad was elevated by thyroxine injection. This result coincides well with previous reports on O_2 consumption [7–9, 24, 25]. In contrast, the activated CO_2 release in the thyroxine-treated male toad was lower than in the normal male toad. This may be due to decreased intensity of locomotor activity caused by thyroxine.

The basal CO_2 release reflects the basal metabolism. Accordingly, our results on basal CO_2 release suggest that the enhancement of basal metabolism by thyroxine is accompanied by a decrease of muscular activity in male toads. This reminds us of the old work that thyroxine leads to the uncoupling of oxidative phosphorylation [27], although this effect was shown to be nonphysiological [28].

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